ASPECTS OF GENE REGULATION IN MAIZE

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Investigations with maize during the past year were designed with several objectives in mind. They ranged from the routine but necessary determination of whether or not a particular gene had come under the control of one of the known gene-control systems, or some yet undefined system, to a more basic examination of the extent and diversity of gene control during development that may be expressed by a single system of controlling elements.

It has long been apparent that a single gene-control system in maize can effect a wide range of expression with respect to time and type of gene action during development. It has been possible to relate some aspects of this diversity to modifications arising solely within one or the other element of a control system. There is now a considerable body of evidence to indicate the presence in higher organisms of different classes of regulatory mechanisms that must integrate with one another in the control of gene expression. Some of these mechanisms effect control of the appearance of the end product of a particular biosynthetic pathway in only some cells of a tissue, even though all cells are known to be competent to express the end product. Their action is reflected in the appearance of distinct patterns of distribution of the end product within different tissues or different parts of an organism.

It is known from early genetic studies that regulation of such patterns may sometimes reside in a single chromosomal locus. The distinctly different distributions of pigment in the elytra of the lady beetle, investigated by C. C. Tan (*Genetics*, 1946), and the distribution of bristles in *Drosophila*, referred to as "step allelomorphism" and discovered many years ago, are evidences of genetic elements that must incorporate a mechanism able to control the activity of particular genes within certain cells of a developing tissue, each allele of the locus being responsible for one pattern. In these

examples, the mechanism comprised within one allele operates independently; whenever two different alleles are present in the nuclei of a developing tissue, each functions to produce its own predetermined distribution of gene product, and the resulting patterns overlap. The isolation of such alleles demonstrates that mutations affecting only the control mechanisms must occur.

Studies with maize have indicated that controlling elements may be solely responsible for this kind of regulation of gene action during development, and that the alleles represent mutants of these elements. The controlling elements behave as independent genetic agents, controlling not only the distribution of a gene product in a developing tissue but also its level or type. Because they regulate the time of expression of particular sets of genes, they serve as "genetic clocks." The different alleles of an element may be said to represent different "settings" of the clock. They arise by mutation, and each mutant is recognized by the marked change it effects in the time or type of gene action during development of a tissue. Plate 1*A* illustrates the distinctiveness of such mutants. In the past these mutations have been referred to as "changes in state." Each state retains its characteristic mode of control of gene action through plant generations unless a subsequent mutation again alters the control mechanism.

The "operator" and "regulator" elements of a control system may function together as component parts of the clocklike mechanism. Combinations, in a zygote or primary endosperm nucleus, of different states of the operator of a system with different states of the regulator can produce a series of widely divergent patterns and types of gene expression in the mature tissue. The controlling elements of a single system, therefore, are exceptionally versatile genetic agents in that they can give rise to a variety of different expressions of a gene during development.

That the controlling elements in maize are even more versatile than was previously suspected will be illustrated in the following sections. Opportunity will be taken first, however, to indicate (table 1) the different instances, examined in the Cold Spring Harbor cultures, in which 7 genes, distributed among 4 different chromosomes of the maize complement, have come under the control of either the *Ac* (Activator) or the *Spm* (Suppressor-

TABLE 1. Examples Studied in the Cold Spring Harbor Cultures of Initiation of Gene Control by the *Ac* and *Spm* Systems

Gene Locus	Symbol for Locus after Inception of Control by the *Ac* or *Spm* System	
	Controlled by the *Ac* System	Controlled by the *Spm* System
Chromosome 3		
A_1	a_1^{m-3}, a_1^{m-4}	a_1^{m-1}, a_1^{m-2},* a_1^{m-5}*
Chromosome 4		
C_2		c_2^{m-1},* c_2^{m-2}
Chromosome 5		
A_2	a_2^{m-4}	a_2^{m-1}, a_2^{m-5}
Chromosome 9		
C_1	c_1^{m-1}, c_1^{m-2}, c_1^{m-3}, c_1^{m-4}	c_1^{m-5}*
Sh_1	sh_1^{m-1}, sh_1^{m-2}	
Bz_1	bz_1^{m-1}, bz_1^{m-2},* bz_1^{m-4}	
Wx	wx^{m-1}, wx^{m-3}, wx^{m-5}, wx^{m-6}, wx^{m-7},* wx^{m-9}*	wx^{m-8}

* Both the operator element and the regulator element were initially at the locus of the gene.

mutator) system. Some of the same genes, as well as others not listed here, have come under the control of other systems, whose modes of operation have not yet been adequately explored.

Parameters of Regulation of Gene Action by the Spm System

A type of *Spm* control examined during the past year illustrates one way in which a "genetic clock" may be set recurrently. The effects of the settings are expressed in the cells of the aleurone layer of the kernels through the control each setting exerts over intensity levels and patterns of distribution of anthocyanin pigment. They are observed in one genetic class of kernels on ears produced by crosses of plants carrying *Spm* and the second of the two modified states of $a_1^{m\text{-}2}$ discussed in *Year Book 62*, where the origin of the state and the initial tests made with it were outlined.

Early studies of this state (state 7977B) indicated the absence of an active *Spm* element at the locus of A_1 although one was present at that locus in the original isolate of $a_1^{m\text{-}2}$. (The A_1 gene in chromosome 3 is involved in the production of anthocyanin pigment in plant and kernel.) The initial plant carrying the modified state had no active *Spm* at any other location in the chromosome complement, but an active *Spm* was introduced by an appropriate cross conducted with one of the two ears of the plant. In kernels of this ear that received from the female parent the modified state of $a_1^{m\text{-}2}$ and from the male parent the standard recessive allele (a_1) and also *Spm*, the expression of gene action resembled that engendered by the original state of $a_1^{m\text{-}2}$. (See legends, pls. 1*B*, 2*A* and *B*.) *Year Book 62* described the results of testcrosses made with plants derived from the $a_1^{m\text{-}2}$–carrying kernels on this ear that had received *Spm* and those that had not received *Spm*. The description placed emphasis on the appearance of a previously unobserved synergistic mode of formation of anthocyanin pigment in the cells of the aleurone layer of some kernels on ears produced by crosses conducted with the plants that had *Spm* in their nuclei. The phenotypes of kernels in this class did not differ on ears produced by reciprocal crosses conducted with the plants. In other words, there was no effect of dose of $a_1^{m\text{-}2}$ on intensity levels or patterns of distribution of anthocyanin in the aleurone layer. Although the early evidence indicated that the kernels with pronounced expressions of this unusual phenotype did not contain an active *Spm*, no such kernels appeared in similar crosses made with sister plants that had no active *Spm* in their nuclei. Neither did they appear on ears produced by reciprocal crosses of these sister plants with plants that were homozygous for standard a_1 and carried one or more active *Spm* elements. These initial observations indicated that *Spm*, present in a plant, is involved in controlling phenotypic expression in progeny kernels that do not receive *Spm*. Experiments conducted this year not only have confirmed that effect but also have revealed still another aspect of regulation of gene action during development.

Among kernels exhibiting the unusual phenotypes, both the patterns of anthocyanin distribution and the range of intensities of pigment among cells of the aleurone layer vary, and are distinctive in each kernel. The maximum pigment intensities range from very high in some kernels to faint in others. The variations are illustrated by several of the kernels on the ear shown in plate 2*A*, and by 5 of the 6 kernels in plate 2*B*. It is evident that the phenotype of each such kernel must arise through some form of control of anthocyanin production and distribution in individual cells during endosperm development, and that the pattern expressed in a mature kernel reflects a "presetting" of the responsible component. It is also evident that this setting must occur before the three haploid nuclei (two from the female and one from the

male) fuse to form the primary endosperm nucleus. Since *Spm* is not present in these nuclei, but must be present in the male or female parent plant carrying state 7977B of a_1^{m-2} in order that pronounced expressions of the unique phenotype may appear, the mechanism responsible for the setting must be activated before *Spm* is removed from spore nuclei as a consequence of the meiotic mitoses. Evidence will be presented below that such activation is independent of the developmental stage and requires only the initial presence of an active *Spm* in the plant.

This year, the types of experiments outlined in *Year Book 62* were repeated on a much larger scale, not only with state 7977B but also with another state of a_1^{m-2} (7995) that resembles it in some aspects of expression. In addition, studies were made to determine the effects of inactive *Spm* and of Spm^w as well as Spm^s in the progeny kernels of plants carrying state 7977B. Plants were grown from a number of kernels that exhibited the unusual phenotypes, and they in turn were subjected to several kinds of test. The results of these studies are summarized below.

Kernels exhibiting pronounced "preset" patterns, similar to those illustrated in plate 2*A* and *B*, appeared on testcross ears of plants carrying an active *Spm*, which might be either Spm^s or Spm^w. (The distinction between the effects produced by Spm^s and Spm^w on the original state of a_1^{m-2} is shown in pl. 1*B*.) All together, 84 plants carrying state 7977B and 28 plants carrying state 7995 were examined. When these plants were crossed with plants that were homozygous for the standard a_1 allele and had no *Spm*, several of them produced ears containing a sector that had developed from a cell in which *Spm* had been removed or inactivated. Within these sectors, nevertheless, some kernels exhibited the pronounced preset patterns of anthocyanin distribution. In one plant, the ear produced by one tiller did not carry active *Spm*, although the ears on the main stalk and on a second tiller did. On the ear in which *Spm* was absent, kernels showing pronounced preset patterns appeared. The range in types of pattern and in maximum pigment intensities among these kernels, and among those within the no-*Spm* sectors of the sectored ears, was as wide as that seen among kernels on the ears of plants that showed no evidence of somatic loss or inactivation of *Spm*. No kernels of the exceptional type appeared, however, on testcross ears produced by 9 plants carrying state 7977B that commenced development with an inactive *Spm*, except within sectors in which *Spm* had changed to its active phase. These tests made it evident that an active *Spm* must be present in a plant, at least initially, to promote the mechanism that is responsible for pronounced preset, synergistically produced anthocyanin patterns in progeny kernels not receiving *Spm*.

Plants were grown from 51 kernels exhibiting such patterns, selected from six ears produced by testcrosses conducted with 3 plants having state 7977B of a_1^{m-2} in chromosome 3 and an active *Spm* in one chromosome 5. Testcrosses designed to detect presence or absence of *Spm* were made with 43 of these 51 plants. (This type of test has been outlined in previous Year Books.) None of the 43 had an active *Spm* element. Plants were also grown from 7 kernels of similar type from an ear of a plant carrying state 7995 of a_1^{m-2} and two active *Spm* elements, neither of which was linked with the a_1^{m-2} locus. No evidence of active *Spm* was found in tests conducted with 5 of the 7 plants.

In order to determine the kinds of pigment patterns that might appear among the kernel progeny of these 58 plants, each was subjected to additional tests. In one test, at least one ear of each plant was utilized as female parent in a cross with a plant that was homozygous for standard a_1 and had no active *Spm*. (Reciprocal crosses were made with only 4 of the plants, but the results were quite

the same.) Ninety-two ears were produced by crosses of this type. With the exception of a very few kernels on ears of plants carrying state 7995, no phenotype resembling that of the kernel from which each plant arose reappeared among the kernels on these ears. Instead, the a_1^{m-2}–carrying kernels were similar to those on one ear, produced by the same type of cross, on the original plant carrying state 7977B. It will be recalled that this plant had no active *Spm*. Such kernels may be nearly colorless; or they may have a few darkly pigmented cells, or areas of faint pigmentation, or both. This expression of state 7977B has continued to reappear in testcrosses conducted with three successive generations of plants (99 plants in all) into which no active *Spm* was introduced. Pigment patterns such as those shown in plate 2*B* have not appeared among the progeny kernels.

A second kind of testcross was conducted with some of the 58 plants derived from the kernels with pronounced preset patterns. One ear each on 24 of the 51 plants carrying state 7977B and on 3 of the 7 plants carrying state 7995 was utilized in a cross with a plant that was homozygous for standard a_1 but had one or more active *Spm* elements. Two classes of a_1^{m-2}–carrying kernels appeared. Kernels that did not receive *Spm* were nearly colorless or had areas of faint pigmentation; their phenotypes were similar to those just described. Kernels that received an active *Spm* had phenotypes resembling that evoked by the original state of a_1^{m-2}. (See, in pl. 2*A*, the Sh_2 kernels with many dots of deep pigment in a lighter background, and in pl. 2*B*, the leftmost kernel, upper row.) No kernels like the other five shown in plate 2*B* appeared on these ears.

The 58 plants just described were derived from kernels with pronounced preset patterns, from ears whose kernel types resembled those seen in plate 2*A*. The photograph shows, in addition to the kernels with preset patterns, two other classes of Sh_2 kernels: those having deeply pigmented spots on a lighter pigmented background (*Spm* present), and those that are colorless or nearly so (no *Spm*). Plants were grown from both types of kernels, selected from the same ears as the kernels from which the 58 plants were derived. From the near-colorless kernels, 28 plants carrying state 7977B and 10 plants carrying state 7995 were grown and tested. From the kernels that had received *Spm*, 32 plants with state 7977B and 19 with state 7995 were grown and examined. The testcrosses made with the 38 plants derived from the near-colorless kernels were like those conducted with the 58 plants grown from kernels having pronounced preset patterns. None of these plants carried an active *Spm*. In crosses with plants homozygous for a_1 and having no *Spm*, none of the progeny kernels exhibited patterns like those shown in plate 2*B*. The a_1^{m-2}–carrying kernels were colorless or nearly colorless. When the testcross introduced an active *Spm*, the a_1^{m-2} locus responded in the expected manner, giving rise to deep spots in a lighter background. In contrast, all 51 of the plants derived from kernels containing *Spm* produced some progeny kernels whose patterns of anthocyanin distribution and intensity levels resembled those shown in plate 2*A* and *B*.

Another set of tests was carried out to reconfirm the conclusion that *Spm* must be present in a plant to initiate the modifications responsible for pronounced preset patterns in some of its no-*Spm* kernel progeny. These tests were made with second-generation progeny of the original plant carrying state 7977B of a_1^{m-2}. One ear of that plant had been produced by a cross with a plant homozygous for standard a_1 and having no active *Spm*; and plants had been grown from the a_1^{m-2}–carrying kernels on that ear. Some of these plants, in turn, were crossed with plants that were homozygous for a_1 but carried one or more active *Spm* elements. Plants were then grown from both *Spm*-carrying and *Spm*-less kernels

produced by three such crosses. Forty-seven plants derived from *Spm* kernels and 20 derived from no-*Spm* kernels were crossed with plants that were homozygous for a_1 and had either no active *Spm* or one or more active *Spm* elements. Once again, kernels exhibiting the unique phenotype appeared on ears of the *Spm*-carrying plants but not on the ears of plants lacking *Spm*.

Thus the results of experiments conducted with three generations of plants carrying state 7977B, and of the more limited but still instructive experiments with plants having state 7995, leave no doubt about the role played by active *Spm* in a plant in controlling the phenotypes of its kernel progeny, both those that receive *Spm* and those that do not. It is also clear that *Spm* does not directly control the presetting mechanism itself, as was shown by the presence, in no-*Spm* sectors on ears of several *Spm*-carrying plants, of kernels with preset patterns. The presetting event, unlike the events that give rise to altered states, does not modify the a_1^{m-2} locus so as to alter the potential for gene expression in a fixed manner. Rather, it appears to involve a release from restrictions, which permits the gene-control mechanism to follow paths that would otherwise be closed. The meaning of "path" in this reference may be clarified by consideration of the distinctive phenotypes of the variegated kernels on the two ears shown in plate 1*A*.

The cross that produced each of these ears was made to test for the presence and number of *Spm* elements in one of the parent plants, which was homozygous for the standard recessive of the gene whose action is exhibited in the kernels on the ear. The other parent had no active *Spm* and was homozygous, in the upper ear, for one particular state of a_1^{m-1}, in the lower ear for a_2^{m-1}. It is known, as a result of many years of testing, that each of these states will respond to a fully active *Spm* by producing a distinctive pattern of anthocyanin distribution in kernels (as shown in the photograph) as well as in the plant. Each of many other isolated states of a_1^{m-1} and a_2^{m-1} also engenders its own precise pattern of anthocyanin distribution in the presence of a fully active *Spm*. States of a_1^{m-1} having expressions similar to that of a_2^{m-1} illustrated here have been isolated, and also states of a_2^{m-1} similar in expression to this state of a_1^{m-1}.

These illustrations are included to emphasize a mode of differential regulation of gene expression among the cells of a tissue. Its control resides in a clocklike mechanism built into the gene-associated element of the control system. In the illustrations given, the clock mechanism was set in motion by the introduction of an activating element, Spm^s, into the primary endosperm nucleus. As long as Spm^s was present in the cells of the developing endosperm, the clock continued to tick off precise events in certain cells at particular stages of development. The end result was a distinctive pattern of distribution of the gene product in cells of the mature tissue.

That cellular environment is not directly involved in control of these regulatory processes was demonstrated, early in the study of the *Spm* system, by crosses between plants that carried different states of a_1^{m-1}. The states were readily distinguished from one another by their markedly different responses to *Spm*. Such crosses produced kernels carrying two different states of a_1^{m-1} as alleles of the A_1 locus. For our purposes, the most illustrative of these crosses combined one state that responds to a fully active *Spm* by producing both large and small areas of light pigmentation with another state that produces only dots of deep pigment, in patterns resembling those seen in the variegated kernels on the upper ear in plate 1*A*. During the development of kernels having both these states and a single Spm^s, each responded to the *Spm* according to its own predetermined ordering of the sequence of events. The patterns and intensities resulting from the individual responses were readily dis-

tinguishable in the aleurone layer, the patterns overlapping each other. In other words, each allele followed its own directed path in the control of A_1 gene action according to a built-in clock mechanism, unaffected by that of the other allele. The resemblance of this phenomenon to the control exerted by different alleles on the pattern of distribution of pigment in the elytra of the lady beetle, mentioned early in this report, is striking.

In the examples just discussed, the clock mechanism is permanently set to follow a definite path during the development of plant or kernel in successive generations, unless a mutation occurs to establish another clock setting. The mechanism is contained in the gene-associated operator element of a two-element system. It may be slowed down, however, or advanced to a maximum rate, according to the activity of the regulator element of the system. Mutations of the regulator can modulate the timing of responses but not their types, which are controlled solely by the operator element. Nevertheless, each allele of an operator responds in the same manner to the timing modulation induced by any one mutant of the regulator. Thus, as was stated earlier, both components of a control system may be involved in the performance of the clock mechanism.

In contrast to permanent settings of the clock, which are responsible for the origin and expression of the states discussed above, settings that are not permanent may occur, as evidenced in part by the observations regarding preset patterns of anthocyanin pigmentation in kernels, described in this report. It has been shown that, although *Spm* is required to initiate the mechanism responsible for these newly detected types of setting, it need not be present in the tissue cells that express the altered settings. Nor do such settings permanently alter the state of the operator, which reverts in the subsequent plant generation to that expressed when it was initially isolated. The impermanent settings may result in a wide range of distinctly different phenotypes, as illustrated by the no-*Spm* kernels shown in plate 2*A* and *B*. The appearance of such a range among the kernel progeny of a single plant indicates that the setting process must occur in a number of cells and that its consequences are not the same in all of them. Once a setting has occurred, however, the phenotype that will be expressed in a kernel receiving the set locus is predestined. The setting will dictate the path of gene activity during kernel development. Thus, the indirect influence of the regulator, *Spm*, in control of the setting process reflects still another parameter of the mode of operation of a gene-control system. It provides one mechanism for inducing widely different patterns of expression of a gene in different parts of an individual organism.

The impermanent settings just mentioned were made evident only in the no-*Spm* kernel progeny of *Spm*-carrying plants. It has been recognized for many years, however, that nonheritable modifications affecting the action of a gene under the control of a known system may occur, and that they may be expressed in cells that do carry the regulator of the system. The component elements responsible for these modifications were not so readily analyzed as those involved in the expression of states 7977B and 7995 of a_1^{m-2}. Each such nonheritable modification is known to arise as the consequence of a single event occurring within a plant cell. The cells derived from the mutant cell carry the modification, whose effects are registered as one type of altered gene action in the progeny cells competent to express this action. Germ cells arising from the same initial cell, however, give no indication that any change has occurred; the modification appears to have been erased in these germ-line cells. In contrast to the preset patterns in kernels, these nonheritable but obviously preset types of gene control are expressed in cells containing an active *Spm* element.

Examples of this kind of modification were mentioned in *Year Book 62*. From such observations it was possible to learn that potential gene action can be set in a cell early in development, so that many cell generations later one particular grade of gene action will be registered in only one kind of tissue produced by the cell's descendants. Some of these nonheritable settings result in a level of gene action resembling that given by the wild-type allele, whereas others effect reduced levels.

The introduction to this report stated that some of the known controlling elements in maize behave as independent genetic agents, in that they contain timing mechanisms for differential control of gene action during development. It also stated that control systems are extraordinarily efficient, a single system having the potentiality to exert a wide range of controls that affect not only times but also types of gene action. The preceding discussions, and those of earlier reports, have attempted to illuminate the many parameters of regulation of gene action that may be ascribed to a single system of controlling elements.

Cyclical Change in Phase of Activity of Ac (Activator)

That the regulator element of the Suppressor-mutator (*Spm*) system may undergo changes in phase of activity was first reported in *Year Book 57*. Only recently was it learned that the regulator element of the Activator (*Ac*) system likewise may undergo changes in phase of activity: from active to inactive and back to active. This discovery was made during an investigation of wx^{m-7}, which is under the control of the *Ac* system. Although wx^{m-7} had been isolated in 1952, from a single kernel on an ear of a plant having a_1^{m-4} (*Ac* system) that was homozygous for the wild-type *Wx* gene, only a relatively few tests had been made with it shortly after its isolation. *Ac* was known to be located, initially, close to the locus of wx^{m-7}. The purpose of the more recent investigation, begun several years ago, was to isolate instances in which *Ac* was removed from this locus but the *Wx* gene remained under the control of the *Ac* system. In the course of the study, the cyclically occurring changes in phase of activity of *Ac* at the wx^{m-7} locus were recognized.

The wx^{m-7} locus was incorporated into plants and kernels carrying various gene markers located not only in the short arm of chromosome 9, where wx^{m-7} resides, but also in other chromosomes of the complement. These combinations brought together in the nuclei of single kernels different gene loci whose action is controlled by the *Ac* system, with the purpose of distinguishing between modifications at the wx^{m-7} locus that affect only *Ac* and those that alter the expression of the *Wx* gene, with or without an accompanying modification in the action of *Ac* through change in its dose or state. A change affecting only *Ac* would be detected by the like response of each of the selected gene loci present in the nuclei of a kernel. Modifications affecting the expression of the *Wx* gene, accompanied or unaccompanied by change in *Ac*, could be identified, individually, by comparison of the expressions of wx^{m-7} with those of the other *Ac*-controlled gene loci in the kernel.

One of the combinations included a particular state of a_1^{m-3}. Gene action at the a_1^{m-3} locus is under the control of the *Ac* system. The selected state produces uniformly light-pigmented plants and kernels in the absence of *Ac* or when *Ac* is present in an inactive phase. When *Ac* is active, this locus gives rise in plants and kernels to well defined areas that are more deeply pigmented or are nonpigmented. Most of these areas consist of descendants of cells in which a mutation has occurred at the a_1^{m-3} locus, and the times and frequencies of such mutations are known to be governed by the state and dose of *Ac* present in the mutating cells. After mutation the action of the

gene is no longer subject to control by the *Ac* system.

Initial evidence of alternating cycles of activity of *Ac* was seen in the kernel progeny of a single plant in a culture of sister plants that were a_1^{m-3} Sh_2/a_1 sh_2; *Ac* wx^{m-7}/wx in constitution. Each of these plants had originated from a kernel whose endosperm contained an active *Ac* element to which both a_1^{m-3} and wx^{m-7} were responding. The plants in this culture were crossed reciprocally with plants that were homozygous for a_1, sh_2, and *wx* and had no *Ac*. Some of them, including the single one mentioned, were also self-pollinated. This particular plant was the only one in which *Ac* had entered an inactive phase. The responsible event must have occurred early in plant development, because an inactive *Ac* was present in the cells that gave rise to the main stalk and tiller; and return to the active phase occurred in only a few cells of the plant.

The state of wx^{m-7} in this plant was one that exhibits an intermediate level of *Wx* gene action with inactive *Ac*, but higher or lower levels when *Ac* is active. With few exceptions, these changes in level of gene action are not the consequence of mutations at the wx^{m-7} locus that result in stable alleles. Subsequent changes continue to occur as long as an active *Ac* is present. This state also has allowed the observation of a previously undetected mode of control of *Wx* gene action during endosperm development. The result is a gradient of levels of *Wx* gene expression in the mature endosperm, which is related to the time of formation of cells during development of the kernel. When kernels are cut longitudinally and the exposed cells stained with an I-KI solution, differential staining of starch in the cells of the endosperm can reveal different levels of *Wx* gene action. Kernels with an inactive *Ac* element have an inner core of cells exhibiting a low level of *Wx* action; and from this core, toward the periphery of the kernel, the level is seen to gradually increase. In kernels having an active *Ac*, on the other hand, some cells within the inner core evince a high level of *Wx* gene action, and some cells nearer the periphery disclose low levels. This mosaicism interrupts the smoothness of the gradient but does not obliterate it.

That *Ac* is inactive in the kernels exhibiting an uninterrupted gradient of *Wx* gene expression is shown by the presence of pale pigment uniformly distributed in the aleurone layer. In particular cells of some kernels, *Ac* has changed from an inactive to an active phase, at times ranging from early to late in the development of the kernel. The descendants of such a cell form a well defined sector, including part of the outer layer of aleurone cells, in which *Ac* activity is registered not only by modified expressions of the *Wx* gene but also by the response of a_1^{m-3}. A pattern of deeply pigmented spots appears in the aleurone-layer area included in the sector, the size of the spots reflecting the time when *Ac* became active. If it did so early in development, the spots may be large; if late, only small dots appear.

Investigation of changes in phase of *Ac* activity at the wx^{m-7} locus was continued this year. One test was conducted to examine the response of the wx^{m-7} locus having an inactive *Ac* to an active *Ac* located elsewhere in the chromosome complement. Reciprocal crosses were made between plants whose constitution was a_1^{m-3} Sh_2/a_1 sh_2; inactive-*Ac* wx^{m-7}/wx and plants that were homozygous for a_1, sh_2, and *wx* and had one active *Ac*. The a_1^{m-3} locus responded to the introduced active *Ac* in accordance with its dose in the endosperm of the kernel. One dose of *Ac* produced early-occurring mutations at the locus; two doses, only late-occurring mutations. The patterns of response were the same in kernels having wx^{m-7} as in those homozygous for the standard *wx* allele. This observation made it evident that the inactive *Ac* at the wx^{m-7} locus was inactive not only with regard to induction of mutation but also with regard to contribution to dose

effects. The wx^{m-7} locus also responded to the introduced active *Ac* by producing changes in *Wx* gene action, at times during endosperm development that corresponded precisely to those of changes at the a_1^{m-3} locus in the same kernel. Evidence has not yet been obtained that the times and frequencies of occurrence of change in phase of *Ac* activity are controlled by a mechanism similar to that observed with *Spm*, but the preliminary findings suggest it.

It must be stressed, in conclusion, that our knowledge of genetic mechanisms that control gene action during development is still too limited to permit the construction of reasonable models at the molecular level to account for the wide range in types of gene control described here and in previous reports. The models of gene regulation that have been proposed to date are not yet fully defined, and cannot be invoked in their present form even though some of their aspects are fitting. There are many parameters of gene control during development. Aspects of each must be clearly defined before an attack at the molecular level can be initiated with hope of success. Awareness of the problems at the genetic level is prerequisite to their solution.

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